Genotypic, Developmental and Drought-Induced Differences in Root Hydraulic Conductance of Contrasting Sugarcane Cultivars[1]

NICANOR Z. SALIENDRA and FREDERICK C. MEINZER[2]

Hawaiian Sugar Planters' Association, P.O. Box 1057, Aiea, Hawaii, USA, 96701

ABSTRACT

Hydraulic properties of entire root systems and isolated roots of three contrasting sugarcane clones were evaluated using transpiration-induced differences in hydrostatic pressure across intact root systems, root pressure-generated xylem sap exudation, and pressure-flux relationships. Regardless of the measurement technique employed, the clones were ranked in the same order on the basis of their leaf area–specific total root system hydraulic conductance (G_{root}). All methods employed detected large developmental changes in G_{root} with maximum values occurring in plants with approximately 0.2 m^2 total leaf area. Genotypic ranking according to G_{root} was reflected as a similar ranking according to root length–specific hydraulic conductance (L) of individual excised roots. Genotypic differences in G_{root} and L were consistent with anatomical characteristics observed in individual roots. Patterns of G_{root} during soil drying and following re-irrigation suggested that the decline in G_{root} observed during soil drying occurred within the roots rather than at the soil–root interface and may have been caused in part by xylem cavitation in the roots.

Key words: Root hydraulic conductance, *Saccharum* spp., transpiration, root pressure, pressure-flux.

INTRODUCTION

An important function of plant root systems is to absorb water from the soil for transport to the shoot. Nevertheless, roots are usually the site of the highest resistance in the pathway for liquid-phase movement of water through the soil–plant–atmosphere continuum (Kramer, 1983). The efficiency of soil water uptake by the root system is, therefore, a key factor in determining the balance between transpiration and shoot water status. Root water transport efficiency is generally expressed as hydraulic conductance, the ratio of water flux through the roots to the water potential gradient between the root xylem and the soil at the root surface (Passioura, 1988; Markhart and Smit, 1990). This gradient has an osmotic and a hydrostatic pressure component, which account for water movement into the roots by osmosis and mass flow, respectively (Fiscus, 1975). Mass flow predominates at high transpiration rates when solute concentrations in the xylem are negligible. Osmotic flow predominates at low transpiration rates when active solute uptake may lead to the development of root pressure and guttation (Fiscus, 1977).

Water movement through the soil–root–shoot continuum follows a radial pathway from the soil–root interface to the root xylem, and an axial pathway from the root xylem to the apoplast of the leaf (Markhart and Smit, 1990). It is widely believed that the Casparian bands of the root endodermis in the radial pathway are the most important barrier to liquid-phase water transport in the entire plant. In the roots of some species, however, e.g. maize and onion, Casparian bands have also been observed in the hypodermis at the outer edge of the cortex (Peterson, 1988). Results of experiments with fluorescent apoplastic dyes suggest that Casparian bands may also be present in the hypodermis of sugarcane roots (Welbaum, unpublished observations).

The physical location of this rate-limiting barrier to water movement is important because of its role in controlling root shrinkage during soil drying, which

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[1] Published as Paper No. 772 in the journal series of the Experiment Station, HSPA.
[2] To whom correspondence should be addressed.

affects soil–root interfacial resistance (Passioura, 1988). If the barrier is in the endodermis, then the water status of the cortex (the tissue likely to shrink) should equilibrate more readily with that of the soil than with that of the xylem. The cortex, therefore, should not shrink as plant water deficits develop because it is in better hydraulic communication with the soil than with the rest of the plant. On the other hand, if the barrier is in the hypodermis, the water status of the cortex should tend to equilibrate with that in the rest of the plant resulting in cortical shrinkage as plant water deficits develop. A large interfacial resistance between the roots and the surrounding soil resulting from root shrinkage at high transpiration rates has been reported in cotton (Huck, Klepper, and Taylor, 1970) and sunflower (Faiz and Weatherley, 1978). In contrast, in wheat (Hansen, 1974a), Italian ryegrass (Hansen, 1974b) and soybean (Blizzard and Boyer, 1980), decreased hydraulic conductivity observed during soil drying was reported to originate within the plant and not in the soil or at the soil–root interface. These contrasting observations may be attributable to differences among species in the location of the rate-limiting barrier to radial movement of water through the roots.

Stomatal conductance is positively correlated with the apparent hydraulic conductance of the soil–root–shoot pathway in a number of species (Aston and Lawlor, 1979; Sanchez-Diaz and Mooney, 1979; Kuppers, 1984; Meinzer, Sharifi, Nilsen, and Rundel, 1988). In sugarcane, root hydraulic conductance and stomatal conductance change in parallel during growth. This developmental coordination of vapour and liquid-phase conductances appears to be mediated by developmental changes in the composition and flux of materials in the xylem sap (Meinzer, Grantz, and Smit, 1991). Similarly, stomatal conductance and soil–root hydraulic conductance are positively correlated among sugarcane genotypes both under well-irrigated conditions and as hydraulic conductance declines during soil drying (Saliendra and Meinzer, 1989).

In view of the linkage between vapour and liquid-phase conductance in sugarcane and other species, understanding of the significance of developmental and drought-induced changes in shoot gas exchange would be improved by more detailed knowledge of the nature of developmental-, genotypic- and drought-associated differences in root hydraulic conductance. In the present study, several independent methods were employed to evaluate root hydraulic conductance of contrasting sugarcane cultivars. The objectives were (i) to assess the validity and utility of each method by comparing the results obtained, (ii) to determine the extent to which drought-induced changes in hydraulic conductance arise within the roots or at the root–soil interface, and (iii) to determine whether geno-

typic differences in hydraulic properties of entire root systems were detectable in individual roots.

MATERIALS AND METHODS
Plant material and growing conditions

Three sugarcane (*Saccharum* spp. hybrid) cultivars were selected on the basis of relative yield in previous salinity and drought resistance tests (Ginoza and Moore, 1985; Ingram, 1985). The cultivars were classified as susceptible (cv. H65–7052), moderately resistant (cv. H67–5630) or resistant (cv. H69–8235) to drought.

Single stem segments containing one lateral bud (seedpiece) were sown in 1 dm^3 plastic pots filled with 4:4:1 (by vol.) mixture of clay soil, commercial potting mix, and volcanic cinders. Plants were grown in the greenhouse and were watered automatically twice daily by drip irrigation. Greenhouse temperatures were typically 30 to 35 °C during the day and 20 to 25 °C at night. Complete fertilizer was first applied one month after planting and once a month thereafter. Plant size rather than age was used as an index of developmental stage. Measurements were carried out on plants with approximately 0·1 to 0·7 m^2 total leaf area. This corresponded to a range in age from 1·5 to 5·5 months. Two kinds of roots develop from sugarcane seedpieces: sett-roots and shoot-roots. Sett-roots are thin and densely branched and originate from the root primordia of the seedpiece, whereas shoot-roots are thick and less branched and arise from root primordia of the basal nodes of the new shoot. One-month-old plants, whose shoot-roots had not yet developed, were used for whole sett-root system measurements. These plants were grown from stem segments sown in 250 cm^3 pots which were fertilized once, two weeks after planting.

Hydraulic properties of entire root systems

Developmental changes in hydraulic properties of entire root systems were evaluated with three independent methods. In intact plants, total root system hydraulic conductance (G_{root}, mmol m^{-2} s^{-1} MPa^{-1}) was estimated from transpiration-induced hydrostatic pressure difference between the soil and the root xylem using an Ohm's Law analogy modified from Meyer and Ritchie (1980):

$$G_{root} = \Delta E / \Delta (\tau - \psi_{CL}) \qquad (1)$$

where E is the transpiration rate per unit leaf area (mmol m^{-2} s^{-1}), τ is the soil water suction (MPa) and ψ_{CL} is the xylem pressure potential (MPa) of a covered, non-transpiring leaf attached at the base of the stalk near the origin of the shoot-roots. ψ_{CL} was taken to represent the root xylem pressure potential (Saliendra and Meinzer, 1989). G_{root} as defined here is a leaf-specific hydraulic conductance (Zimmermann, 1978) obtained as the slope of a line fitted by linear regression to a plot of E on the ordinate versus $\tau - \psi_{CL}$ (Fig. 1A). E, τ, and ψ_{CL} were measured at hourly intervals from 0800 h to 1500 h on clear days in the greenhouse. E was determined gravimetrically by sealing pots in plastic bags, then recording weights to the nearest 0·1 g and time intervals to the nearest second. For plants too large to weigh conveniently, *E was calculated from the mass flow rate of sap measured with stem heat balance gauges (Dynamax Inc., Houston, TX, USA)* linked to a data logger (21X with AM32 multiplexer, Campbell Scientific Inc., Logan, UT, USA). τ was measured with tensiometers (model R106, Irrometer Inc., Riverside, CA, USA) installed with their tips near the bottom of the pots where most of the roots were located. ψ_{CL} was measured with a pressure chamber (PMS Inc., Corvallis, OR, USA) using laminar segments of leaves (Salien-

dra, Meinzer, and Grantz, 1990) covered with aluminium foil on the previous day.

After determination of G_{root} with the transpiration-induced pressure difference method described above, G_{root} was estimated using root pressure-generated flow. The plants were detopped prior to dawn by cutting through the stem at approximately 10 cm above the soil surface with a sharp razor blade. Green leaf blades were excised at their ligule and immediately sealed in long plastic bags for subsequent measurement of their area with a leaf area meter (model 3100, LI-COR Inc., Lincoln, NE, USA). This permitted E and G_{root} to be expressed on a unit leaf area basis and allowed total leaf area to be used as an index of plant developmental stage. The detopped root systems were irrigated and immediately brought to the laboratory where the remaining stems were fitted with plastic collars for collection of natural root pressure exudate. Xylem sap was collected at 10 min intervals and weighed to the nearest 0·1 mg to determine flow rate. Steady-state flow rates under root pressure were typically maintained for several hours, but only xylem sap collected between 10 and 60 min was weighed and stored at $-80\,°C$ for subsequent determination of osmolality with a vapour pressure osmometer (Model 5100 B, Wescor Inc., Logan, UT, USA). Sap osmotic potential (ψ_π) at 25 °C was calculated by multiplying (mmol kg^{-1}) by 0·00248 MPa mmol^{-1} kg. For the purpose of calculating the driving force for water movement under conditions of root pressure-induced flow, the ψ_π of the soil solution was taken as zero, the approximate ψ_π of water draining from the pots after irrigation. This permitted G_{root} to be calculated by dividing the root pressure exudation rate (J_V) by the ψ_π of the exudate:

$$G_{root} = J_V / \psi_\pi. \qquad (2)$$

After measurement of root pressure-generated xylem exudation rates, the stump of the cut stem was inserted through a hole in the centre of a rubber stopper and the entire pot containing the intact root system was sealed inside a specially constructed 18·5 dm^3 cylindrical pressure vessel for determination of G_{root} from pressure-flux relationships. The protruding stump of the stem was fitted with a plastic collar for collection of xylem exudate and the entire root system was pressurized with nitrogen gas initially at approximately 0·15 MPa. The pressure was raised in 0·15 MPa increments until a final pressure of 0·60 MPa had been attained. Xylem exudate was collected every 3 to 5 min and transferred to a pre-weighed vial, which was reweighed immediately to the nearest 0·1 mg. Approximately 20 min were required to attain a steady-state flow rate at each pressure applied. Linear increase in flow with pressure and stability of flow for at least 40 min at each pressure, suggested that use of nitrogen gas did not induce hypoxia. Air already present in the soil and in the pressure vessel before pressurization, negligible leakage from the vessel, and the relatively short period (*c.* 2 h) during which the roots were pressurized probably contributed to avoidance of hypoxia. G_{root} was calculated as the slope of the line fitted by linear regression to a plot of flux on the ordinate axis versus pressure applied (Markhart and Smit, 1990):

$$G_{root} = \Delta J_V / \Delta P \qquad (3)$$

where P is the pressure applied.

The pressure-flux technique described above was also used to characterize the hydraulic properties of sett-root systems. One-month-old plants were removed from their small pots. The root systems were washed carefully with water and immediately placed in cylindrical plastic containers filled with 25% Hoagland solution. The plants were then detopped by cutting the stem 3 cm above the shoot–root axis and the stump of the cut stem

was inserted through a hole in the centre of a rubber stopper. The root system-stopper assemblies were sealed inside a specially constructed ten-chamber apparatus which was pressurized with compressed air in increments of 0·05 MPa from 0·05 to 0·25 MPa. The solution and the roots were aerated during measurements. Xylem exudate was collected in small vials filled with tissue paper weighed to the nearest 0·1 mg before and after each sap collection. An inverted beaker lined with moist filter paper was placed over the vial and cut stem to prevent evaporation from the tissue paper between weighings. Preliminary measurements indicated that steady-state flow rates were attained at approximately 10 min after applying each new pressure. Upon completion of the pressure-flux measurements, total lengths of the sett-root systems were measured with an area meter (Delta-T Area Meter System, Delta-T Devices, Cambridge, UK), set on a length mode, permitting flux and hydraulic conductance to be expressed on a unit root length basis. Root length–specific hydraulic conductance (L, mmol m^{-1} s^{-1} MPa^{-1}) was calculated as the slope of the pressure-flux relationships as described above.

Changes in G_{root} during soil drying and following re-irrigation were monitored with the pressure-flux and transpiration-induced hydrostatic pressure difference techniques. Soil drying was monitored with tensiometers and irrigation was resumed when soil suction reached 0·07 MPa, a value previously determined to cause substantial reductions in G_{root} and stomatal conductance in greenhouse-grown sugarcane (Saliendra and Meinzer, 1989). Since determination of G_{root} with the pressure-flux method required detopping, different sets of plants were used before and during soil drying, and following re-irrigation. G_{root} of intact plants was calculated as the ratio of E to soil–root hydrostatic pressure difference (τ-ψ_{CL}), and G_{root} of detopped root systems was calculated as the flux divided by 0·4 MPa, the single pressure applied. G_{root} calculated by the former and the latter thus may not be identical to G_{root} calculated from the slopes of transpiration-pressure difference and pressure-flux relationships, respectively.

Hydraulic properties of isolated roots

Individual shoot-roots were obtained by removing the entire root–soil mass from the pot exposing the white, active shoot-roots located along the sides and the bottom of the pot. White shoot-roots approximately 2 mm in diameter were rapidly selected and excised at 8 cm from their tip. Excised roots were immediately placed in a 25% Hoagland solution and brought to the laboratory for determination of L with the pressure-flux method. Individual roots were prepared for pressurization by inserting the cut end of a root through a small hole in the centre of a rubber stopper with the aid of an insertion tool. The same apparatus and procedures were used as described above for sett-root system measurements.

Exudate was collected at 2 to 3 min intervals for 10 to 15 min after steady-state flow rates were attained. A new set of roots was used at each pressure increment. Flux was expressed on a unit root length basis. L was calculated as the slope of a line fitted by linear regression as described above.

Root anatomy

Individual shoot-roots of the three sugarcane cultivars were selected and excised near the tip as described in the preceding section. Anatomical specimens were prepared as described by Luza and Polito (1988). Briefly, excised roots were fixed with 4% glutaraldehyde in 0·05 M phosphate buffer, pH 6·8, and dehydrated through an ethanol series. Samples were embedded in glycol methacrylate (Sorvall) and sectioned at 3 μm with glass

knives on a Sorvall JB-4 microtome. Sections were stained with aniline blue-black, periodic acid–Schiff's reaction, and decolorized aniline blue.

Cross-sectional diameters of the stele and the entire root were measured under a light microscope, and their cross-sectional areas were calculated. The area of the cortex (A_{cortex}) was calculated from the difference between the entire root area and the stele area (A_{stele}). In addition, radii and areas of individual metaxylem elements were determined from stele photographs using a digitizer (Sigma-Scan, Jandel Scientific, Corte Madera, CA, USA) linked to a personal computer.

RESULTS

The hydrostatic pressure difference between the soil and the root xylem was linearly related to the transpirational flux of water in intact sugarcane plants whose leaf areas were in the range of 0·2 to 0·3 m² plant⁻¹ (Fig. 1A). There were consistent differences among cultivars in the relation-
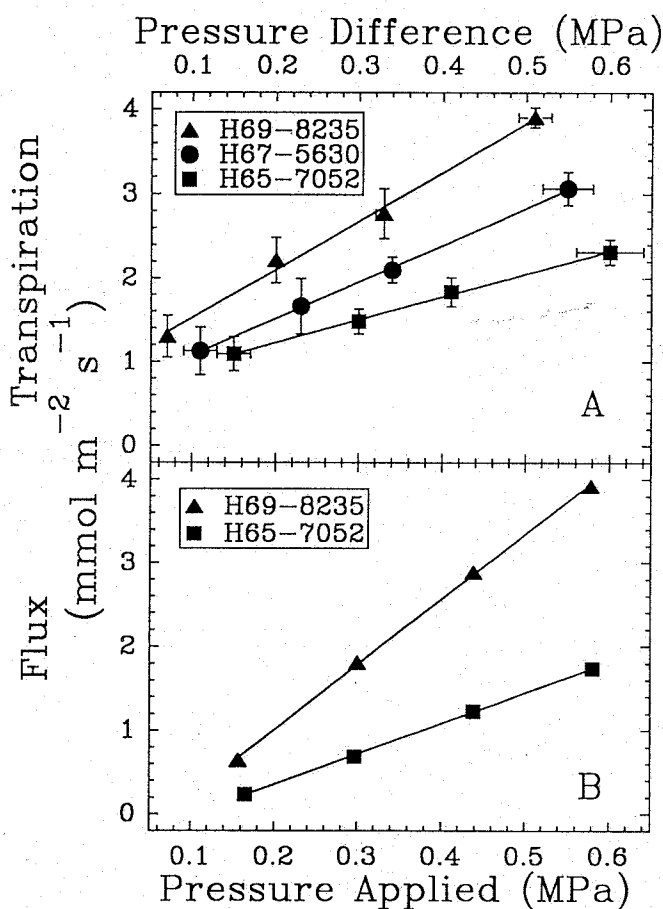
ship between transpiration rate and hydrostatic pressure difference. At a given transpiration rate, the ranking of cultivars according to the pressure difference generated was H65–7052 > H67–5630 > H69–8235. Therefore, the root system of cv. H69–8235 was able to sustain a greater transpirational flux of water at a given hydrostatic pressure difference between the bulk soil and the root xylem.

When the pressure-flux technique was used to determine the hydraulic properties of detopped root systems of cv. H65–7052 and cv. H69–8235, patterns similar to those observed with the transpiration-pressure difference technique were obtained with plants whose leaf areas ranged from 0·6 to 0·8 m² plant⁻¹ (Fig. 1B). At all pressures applied, water flux from roots of cv. H69–8235 was greater than that from roots of cv. H65–7052. For both cultivars, water flux at a given pressure applied (Fig. 1B) was always less than the transpiration rate at a corresponding soil–root hydrostatic pressure difference (Fig. 1A). However, the slopes of the pressure-flux relationships were steeper than those of the transpiration-pressure difference relationships indicating that the pressure-flux method yielded higher estimates of G_{root} than the transpiration-pressure difference method (Table 1). Regardless of the method employed, cv. H69–8235 exhibited the highest values of G_{root} which were roughly twice the values obtained for cv. H65–7052.

The pressure-flux technique was also used to characterize the hydraulic properties of individual excised shoot-roots and whole sett-root systems (Fig. 2). At a given applied pressure, the water flux per unit root length was highest in cv. H69–8235 and lowest in cv. H65–7052. The ranking among cultivars with respect to the pressure-flux relationships was thus the same regardless of whether entire root systems (Fig. 1), individual shoot-roots (Fig. 2A), or entire sett-root systems (Fig. 2B) were used. However, water fluxes per unit root length (Fig. 2) and the corresponding hydraulic conductances (Table 2) were approximately an order of magnitude greater in excised shoot-roots than those in the whole sett-root systems.

FIG. 1. (A) The relationship between transpiration rate (E) and hydrostatic pressure difference between the soil and the root xylem (τ-ψ_{CL}) in intact, well-irrigated plants of three sugarcane cultivars whose size ranged from 0·2 to 0·3 m² leaf area plant⁻¹. Data points are means ($\pm$ standard error) of five to ten observations from four plants of each cultivar. (B) Xylem exudate flux in relation to applied pressure for whole root systems of detopped, well-irrigated plants of two sugarcane cultivars whose size ranged from 0·6 to 0·8 m² leaf area plant⁻¹. Fluxes are expressed per unit leaf area of the excised shoot. Data points are means ($\pm$ standard error) of ten observations from two root systems of each cultivar. Standard errors are smaller than the symbols.

TABLE 1. *Leaf area-specific hydraulic conductance of entire root systems* (G_{root}) *determined with the transpiration-pressure difference and pressure-flux techniques using well-irrigated plants of three sugarcane cultivars*

G_{root} is the slope of lines fitted to the data in Fig. 1 by regression. Plant sizes ranged from 0·2 to 0·3 and from 0·6 to 0·8 m² leaf area plant⁻¹, for the transpiration-pressure difference and pressure-flux techniques, respectively. G_{root} estimates within a column followed by the same letter are not significantly different by Tukey's studentized range (*HSD*) test ($\alpha = 0.05$, df = 6 for $\Delta E/\Delta(\tau - \psi_{CL})$ and df = 4 for $\Delta J_v/\Delta P$).

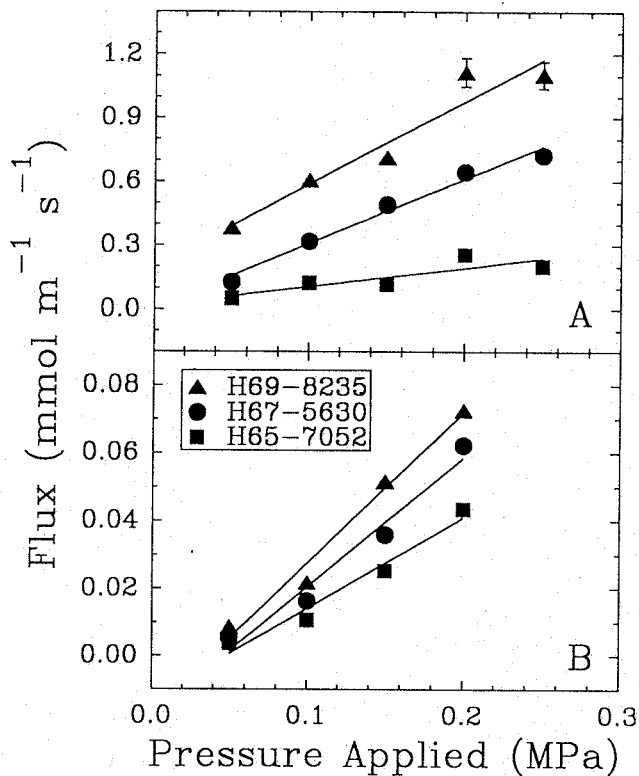| Cultivar	G_{root} (mmol m⁻² s⁻¹ MPa⁻¹)	
	$\Delta E/\Delta(\tau - \psi_{CL})$	$\Delta J_v/\Delta P$
H69-8235	5·76 a	7·78 a
H67-5630	4·39 b	—
H65-7052	2·70 c	3·64 b

FIG. 2. Xylem exudate flux per unit root length in relation to applied pressure for (A) single, excised shoot-roots and (B) whole sett-root systems of three sugarcane cultivars. Data points are means (±standard error) of nine observations from three shoot-roots or sett-root systems of each cultivar. Most of the standard errors are smaller than the symbols.

Shoot-roots used were excised at 8 cm from their tips, whereas sett-root systems contained roots in the range of 20 to 40 cm long. The larger proportion of immature tissue in shoot-roots, therefore, may have contributed to their higher hydraulic conductance on a unit length basis (Table 2).

Anatomical measurements of shoot-roots at approximately 8 cm from the tip revealed that the area of the stele and the A_{stele}/A_{cortex} ratio of cv. H69–8235 were greater than those for the other two cultivars (Table 3). The average metaxylem element radius and the total

TABLE 2. *Root length-specific hydraulic conductance (L) of single, excised shoot-roots and entire sett-root systems determined with the pressure-flux technique using well-irrigated plants of three sugarcane cultivars*

L is the slope of the line fitted to the data in Fig. 2 by regression. L estimates within a column followed by the same letter are not significantly different by Tukey's studentized range (HSD) test ($\alpha = 0.05$, df = 6).

Cultivar	L (mmol m^{-1} s^{-1} MPa^{-1})	
	Shoot-roots	Sett-roots
H69-8235	3·90 a	0·44 a
H67-5630	3·03 b	0·38 b
H65-7052	0·88 c	0·27 c

metaxylem area were also larger in cv. H69–8235 than in the other two cultivars (Table 3). The A_{stele}/A_{cortex} ratio serves as a tortuosity index for the radial flow of water through the root tissue. Thus, though cv. H67–5630 had the greatest external surface area for absorption, it also had the most tortuous water flow from the root surface through the vascular bundles as suggested by its lowest value of A_{stele}/A_{cortex}. These genotypic differences in radial and axial anatomical characteristics were consistent with the genotypic variation in G_{root} shown in Tables 1 and 2.

Considerable developmental changes in G_{root} were detected with all three independent methods employed to evaluate hydraulic properties of entire root systems (Figs 3, 4). With the transpiration-induced pressure difference technique (Fig. 3A), G_{root} increased to a maximum value at a plant size of approximately 0·2 m² leaf area plant^{-1}, then declined precipitously up to approximately 0·3 m² leaf area plant^{-1} beyond which it declined more slowly with further increase in plant size. Cultivar H69–8235 exhibited significantly higher G_{root} than cv. H65–7052 in plants larger than 0·2 m² leaf area. Similar developmental changes in G_{root} were observed with the pressure-flux technique (Fig. 3B), but with maximum G_{root} in cv. H69–
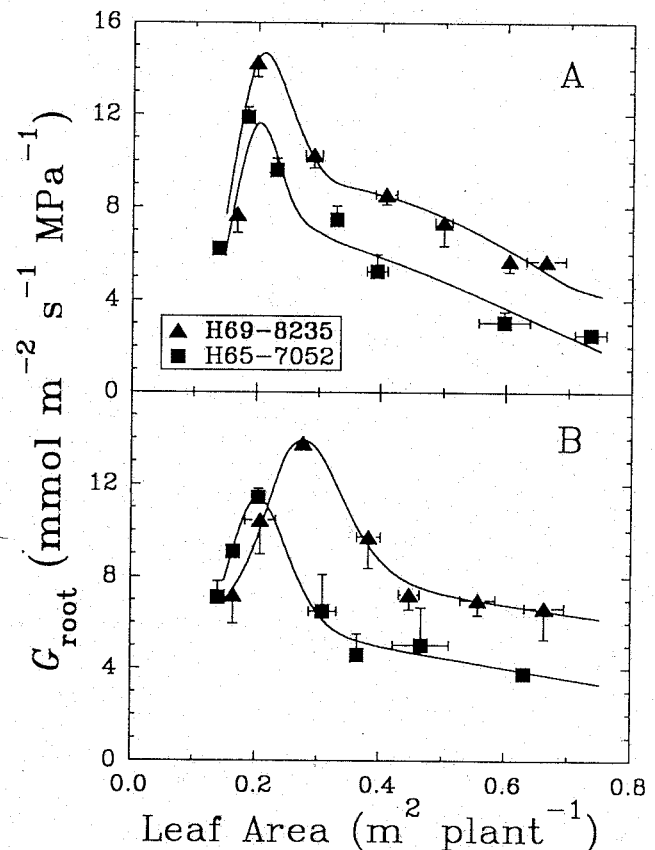

FIG. 3. Leaf area-specific hydraulic conductance of entire root systems (G_{root}) as a function of total leaf area. G_{root} was calculated from (A) equation (1) and (B) equation (3) given in text. Data points are means (±standard error) of two to four plants.

TABLE 3. *Anatomical characteristics determined from cross-sections of shoot-roots excised from well-irrigated plants of three sugarcane cultivars*

Data are means (±standard error) of 13 to 17 observations.

Cultivar	Radial characteristics			Metaxylem (axial) characteristics		
	A_{stele} (mm^2)	A_{cortex} (mm^2)	A_{stele}/A_{cortex}	Number	Radius (μm)	Total area $(10^5 \ \mu m^2)$
H69-8235	1·33(±0·34)	3·56(±0·89)	0·37(±0·02)	11·2(±1·3)	64·3(±3·8)	1·46(±0·22)
H67-5630	1·18(±0·19)	5·70(±0·94)	0·21(±0·02)	13·9(±0·9)	51·9(±4·6)	1·18(±0·21)
H65-7052	0·52(±0·05)	2·03(±0·26)	0·26(±0·02)	9·5(±0·7)	58·2(±2·2)	1·01(±0·15)

8235 being measured at a plant size of approximately 0·28 m^2 leaf area plant^{-1}.

The pattern of developmental variation in G_{root} determined from root pressure-generated xylem exudation rates (Fig. 4C) was similar to those obtained when G_{root} was determined at higher transpiration- and pressure-induced flow rates (Fig. 3). However, absolute values of G_{root} determined with the root pressure method were approximately an order of magnitude lower than those obtained with the other two methods. Generally, cv. H69-8235 had higher xylem exudation rates than cv. H65-7052 (Fig. 4A), but osmolalities of the xylem exudate (Fig. 4B) were similar in the two cultivars over the range of plant size used. Consequently, G_{root} (calculated as the ratio of flow rate to sap osmotic potential) of cv. H69-8235 was generally higher than cv. H65-7052 (Fig. 4C), indicating that genotypic differences in G_{root} determined with this method were attributable to factors other than root xylem osmotic potential.

The effect of a soil drying and re-irrigation cycle on G_{root} was assessed with both the pressure-flux and transpiration-pressure difference techniques. Soil drying to 0·07 MPa soil suction caused reductions of 50 to 80% in G_{root} assessed with the pressure-flux technique (Table 4). Within 1 h after re-irrigation, G_{root} of cv. H65-7052 had recovered completely and that of cv. H69-8235 had recovered to more than 80% of its initial value (Table 4). In apparent contrast, 24 to 72 h were required for full recovery of G_{root} following re-irrigation when it was measured by the transpiration-pressure difference tech-

TABLE 4. *Leaf area-specific hydraulic conductance of entire root systems* (G_{root}) determined with the pressure-flux technique using detopped plants of two sugarcane cultivars subjected to three irrigation treatments

G_{root} was calculated as the ratio of flux to the constant pressure applied (0·4 MPa). Data are means (±standard error) of 18 to 24 observations from three to four root systems of each cultivar.

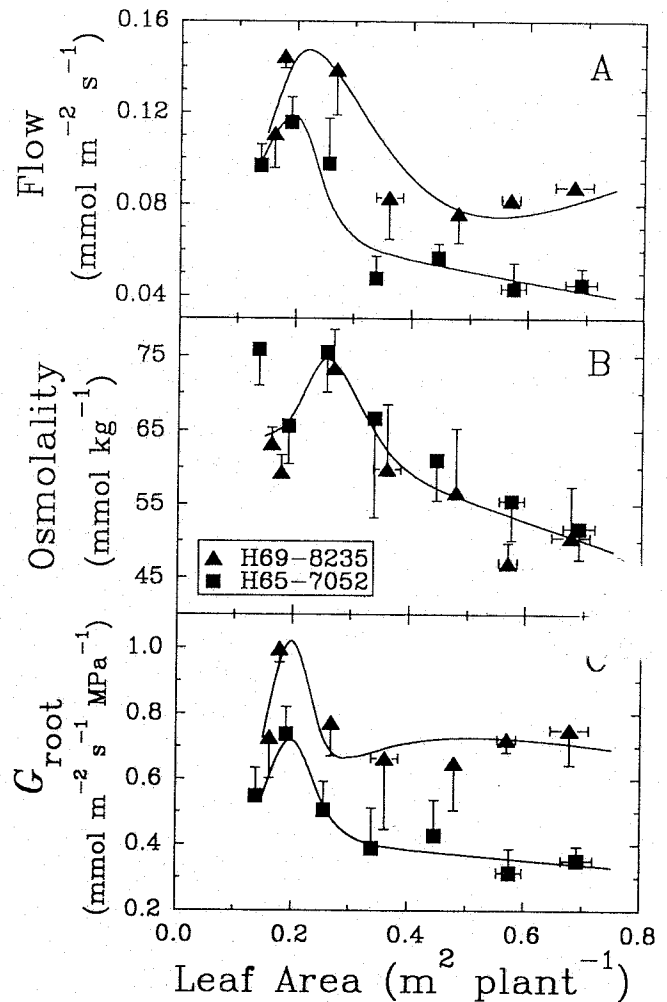| Treatment	G_{root} (mmol m^{-2} s^{-1} MPa^{-1})	
	H65-7052	H69-8235
Irrigated	2·9(±0·1)	6·3(±0·2)
Droughted	0·6(±0·1)	3·2(±0·3)
Re-irrigated	3·4(±0·1)	5·2(±0·3)

FIG. 4. Properties of detopped root systems as a function of plant size. (A) Root pressure-generated xylem sap flow rate on a unit leaf area basis; (B) osmolality of xylem sap and (C) root hydraulic conductance (G_{root}) calculated from equation (2) in text. Data points are means (±standard error) of three to six plants.

nique in intact plants even though root xylem pressure potential (ψ_{CL}) recovered fully within 1 h (Fig. 5).

DISCUSSION

The higher values of G_{root} obtained from pressure-flux relationships than from transpiration-pressure difference relationships (Table 1) may be attributable to differences
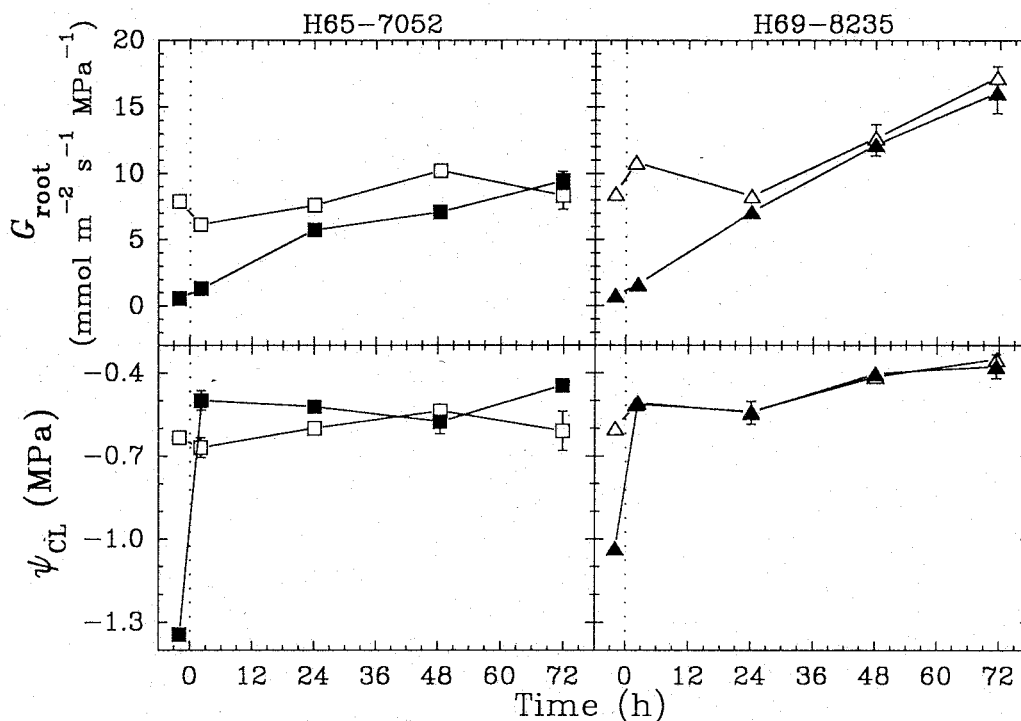
FIG. 5. Time-courses of root hydraulic conductance (G_{root}) and root xylem pressure potential (ψ_{CL}) for well-irrigated sugarcane plants (open symbols) and plants growing in soil dried to a soil suction of 0·08 MPa and then re-irrigated at 0 h (closed symbols). G_{root} was calculated as the ratio, $E/(\tau - \psi_{CL})$. Data points are means (±standard error) of three to five observations from four plants of each cultivar.

in the nature of the driving forces and pathways involved in the transpiration- and pressure-induced movement of water through the root systems of intact and detopped plants, respectively. Evidence that pressure-induced water flow through roots can follow a route different from that of transpiration-induced flow has been provided by other studies. Koide (1985) reported that formerly air-filled spaces were added as a new flow path of water transport when roots were pressurized, while Salim and Pitman (1984) demonstrated that significant amounts of water movement occurred in the cortex when pressure was applied to detopped root systems. Despite these possible artifacts associated with the pressure-flux technique, it was reliable for characterizing genotypic and developmental differences in root hydraulic properties among sugarcane cultivars (cf. Fig. 3A and B).

Several factors may have contributed to the lower values of G_{root} obtained with the root pressure-generated flow technique. Among these are the inability to measure the water potential gradient accurately, and the non-linear relationship between the hydrostatic pressure gradient and water flux when the pressure gradient is small (Markhart and Smit, 1990). In addition, removal of the shoot may have reduced osmotically-driven water flow by inhibiting the transport of carbohydrates from the shoot to the root (Bowling, Watson, and Ehwald, 1985). Despite these potential limitations in sugarcane and other species exhibiting root pressure, the root pressure-

generated flow method is easy to use, requiring no special apparatus with the exception of an osmometer, and yields patterns of genetic and developmental variation in G_{root} similar to those obtained with the other two techniques employed in this study.

When the pressure-flux technique was applied to individual excised shoot-roots (Fig. 2A), genotypic differences in G_{root} were consistent with those obtained with entire root systems (Fig. 1), suggesting that differences among cultivars in whole root system transport properties were attributable to variations in intrinsic hydraulic properties of single roots rather than gross morphological features such as total root system length and degree of branching. The data presented in Table 3 suggest that genotypic differences in hydraulic properties of single roots may have had an anatomical basis. For example, the higher total metaxylem area in cv. H69–8235 would have contributed to higher axial conductance. Radial conductance was also probably higher in this cultivar because its higher A_{stele}/A_{cortex} ratio suggested that the radial movement of water through its root tissue was more efficient, and its higher A_{stele} denoted greater stelar circumference, providing a greater endodermal surface area per unit root length for water influx than the other two cultivars. These anatomical differences do not rule out additional genotypic differences in membrane permeability in both the endodermis and hypodermis.

The large difference in L between shoot-roots and sett-

roots (Table 2) could have resulted from substantial differences in their axial conductances. If the xylem vessels are treated as circular tubes with constant diameter, according to the Poiseuille equation, the axial conductance of the root is directly proportional to the number of conducting xylem elements and to the fourth power of their radii (Frensch and Steudle, 1989). Based on visual observations, the diameter of the sett-roots was estimated to be 0·10 to 0·25 times the diameter of the shoot-roots. Although this may imply a greater radial conductance in the sett-roots, a greater number of xylem elements with much larger radii in the shoot-roots probably determined the observed differences in the total hydraulic conductance between these two types of root. A very conservative estimate of just a 2-fold difference in the radii of xylem elements between sett-roots and shoot-roots would have resulted in axial flow rates of the shoot-roots that were 16 times greater than those for the sett-roots at a given pressure difference.

Decreases in the hydraulic conductance of root systems subjected to drought have been attributed to xylem cavitation in cotton (Byrne, Begg, and Hansen, 1977), decreased cell permeability in lemon and citrus (Ramos and Kaufmann, 1979; Levy and Syvertsen, 1983), rectifier-like activities in desert succulents (Nobel and Sanderson, 1984), and increased resistance at the soil–root interface (Faiz and Weatherley, 1978). In the present study, the slow recovery of G_{root} measured by the transpiration-pressure difference technique upon re-irrigation of droughted intact plants (Fig. 5) suggested that in sugarcane, changes in hydraulic properties within the root system, rather than at the soil–root interface occurred in response to soil drying. However, when G_{root} was measured by pressurizing detopped root systems, its recovery was rapid upon re-irrigation of droughted plants (Table 4). This discrepancy in the rates of recovery of G_{root} measured by the transpiration-pressure difference and pressure-flux methods can be explained if the decline in G_{root} during soil drying was caused by embolism formation. Recovery from embolism formation would likely be much slower if the driving force for water movement were transpirational pull than if it were external pressurization. Schultz and Matthews (1988) and Tyree, Fiscus, Wullschleger, and Dixon (1986) have suggested that formation of embolism in stems may be a common occurrence in both droughted and irrigated plants and that the root pressure generated by the loading of solutes into the root xylem may be important in dissolving emboli and in refilling of vapour-filled vessels during the night. Therefore, it is also possible that a more immediate role of root pressure is in the repair of drought-cavitated xylem in the roots themselves.

All methods employed in the present study pointed to large developmental changes in leaf area-specific root hydraulic conductance in sugarcane. Substantial develop-

mental changes in root hydraulic properties have also been noted in other species (Fiscus and Markhart, 1979). Given the close developmental co-ordination between stomatal conductance, transpiration and plant hydraulic properties previously demonstrated in sugarcane (Meinzer et al., 1991; Meinzer and Grantz, 1991), these factors should be considered in interpreting patterns of shoot gas exchange and their genotypic variation in other species.

ACKNOWLEDGEMENT

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